

WHAT IS CLAIMED IS:

1. A process for linearly amplifying a specific nucleic acid sequence comprising the steps of:

providing:

said specific nucleic acid sequence,

an initial primer or a nucleic acid construct comprising two segments,

(A) a first segment (i) being substantially complementary

to a first portion of said specific nucleic acid sequence and (ii)

capable of template-dependent first extension, and

(B) a second segment being (i) substantially non-identical

to said first segment, (ii) substantially identical to a second

portion of said specific nucleic acid sequence, (iii) capable of

binding to a complementary sequence of said second segment

and (iv) capable of providing for subsequent binding of a first

segment of a second primer or nucleic acid construct to said

first portion of said specific nucleic acid sequence under

isostatic or limited cycling conditions, such that a second primer

extension is produced and displaces a first primer extension;

and

substrates, buffer and a template-dependent polymerizing

enzyme; and

incubating said specific nucleic acid sequence and said novel primer or nucleic acid construct in the presence of said substrates, buffer and template-dependent polymerizing enzyme under isostatic or limited cycling conditions; thereby linearly amplifying said specific nucleic acid sequence.

2. The process of claim 1, wherein said initial primer or nucleic acid construct and said second primer or nucleic acid construct are the same.
3. The process of claim 1, wherein said initial primer or nucleic acid construct and said second primer or nucleic acid construct are different.
4. The process of claim 1, wherein said first segment or said second segment or said primer extension, or any of the foregoing, comprise at least one modified nucleotide or nucleotide analog.
5. The process of claim 4, wherein said second segment comprises at least one modified nucleotide or nucleotide analog which increases the thermodynamic stability of said first segment to its complement in said primer extension.
6. The process of claims 4 or 5, wherein said modified nucleotide or nucleotide analog comprises an intercalating agent.
7. The process of claim 1, wherein said first segment or said primer extension or both comprises at least one modified nucleotide or nucleotide analog.
8. The process of claim 7, wherein said modified nucleotide or nucleotide analog decreases the thermodynamic stability of said first segment or said primer extension to its complement.
9. The process of claim 8, wherein said modified nucleotide or nucleotide analog comprises a negatively charged chemical group.

10. The process of claim 9, wherein said negatively charged chemical group comprises carboxylic acid.

11. The process of claim 1, wherein said initial primer or nucleic acid construct, or said second primer or nucleic acid construct, or both, comprises a nucleic acid selected from the group consisting of a linear nucleic acid, branched nucleic acid, an inverted nucleic acid and a peptide-nucleic acid, or a combination of any of the foregoing.

12. A process for non-linearly amplifying a specific nucleic acid sequence comprising the steps of:

providing:

 said specific nucleic acid sequence,
 a first initial primer or a nucleic acid construct for said specific nucleic acid sequence, said first initial primer or nucleic acid construct comprising two segments:

 (A) a first segment (i) being substantially complementary to a first portion of said specific nucleic acid sequence and (ii) capable of template-dependent first extension, and

 (B) a second segment being (i) substantially non-identical to said first segment, (ii) substantially identical to a second portion of said specific nucleic acid sequence, (iii) capable of binding to a complementary sequence of said second segment and (iv) capable of providing for subsequent binding of a first segment of a second primer or nucleic acid construct to said first portion of said specific nucleic acid sequence under isostatic or limited cycling conditions, such that a second primer extension is produced to displace a first primer

extension; and

a subsequent initial primer or a nucleic acid construct to the complement of said specific nucleic acid sequence, said subsequent initial primer or nucleic acid construct comprising two segments,

(A) a first segment (i) being substantially complementary to a first portion of said specific nucleic acid sequence and (ii) capable of template-dependent first extension, and

(B) a second segment being (i) substantially non-identical to said first segment, (ii) substantially identical to a second portion of said specific nucleic acid sequence, (iii) capable of binding to a complementary sequence of said second segment and (iv) capable of providing for subsequent binding of a first segment of a subsequent primer to said first portion of said specific nucleic acid sequence under isostatic or limited cycling conditions, such that a second primer extension is produced and displaces a first primer extension; and substrates, buffer and a template-dependent polymerizing enzyme; and incubating said specific nucleic acid sequence and said novel primer or nucleic acid construct in the presence of said substrates, buffer and template-dependent polymerizing enzyme under isostatic or limited cycling conditions; thereby non-linearly amplifying said specific nucleic acid sequence.

13. The process of claim 12, wherein said first initial primer or nucleic acid construct and said second initial primer or nucleic acid construct are the same.

14. The process of claim 12, wherein said first initial primer or nucleic acid construct and said second initial primer or nucleic acid construct are different.

15. The process of claim 12, wherein at least one member selected from the group consisting of said first segment or said second segment of the first initial primer or nucleic acid construct, said first segment or said second segment of the second initial primer or nucleic acid construct, and said primer extension, or any of the foregoing, comprise at least one modified nucleotide or nucleotide analog.
16. The process of claim 15, wherein the second segment of the first initial primer or the second initial primer or both comprises at least one modified nucleotide or nucleotide analog which increases the thermodynamic stability of said first segment to its complement in said primer extension.
17. The process of claims 15 or 16, wherein said modified nucleotide or nucleotide analog comprises an intercalating agent.
18. The process of claim 12, wherein said first segment of the first initial primer or said first segment of the second initial primer, or both, or their primer extension, or any combination thereof, comprises at least one modified nucleotide or nucleotide analog.
19. The process of claim 18, wherein said modified nucleotide or nucleotide analog decreases the thermodynamic stability of said first segment or said primer extension, or both, to its complement.
20. The process of claim 19, wherein said modified nucleotide or nucleotide analog comprises a negatively charged chemical group.

21. The process of claim 20, wherein said negatively charged chemical group comprises carboxylic acid.

22. The process of claim 12, wherein said first initial primer or nucleic acid construct, or said second initial primer or nucleic acid construct, or both, comprises a nucleic acid selected from the group consisting of a linear nucleic acid, branched nucleic acid, an inverted nucleic acid and a peptide-nucleic acid, or a combination of any of the foregoing.

23. A process for non-linearly amplifying a specific nucleic acid sequence comprising the steps of:
providing:
said specific nucleic acid sequence and its complement;
a first initial primer or a nucleic acid construct for said specific nucleic acid sequence, said first initial primer or nucleic acid construct comprising two segments:
(A) a first segment (i) being substantially complementary to a first portion of said specific nucleic acid sequence and (ii) capable of template-dependent first extension, and
(B) a second segment being (i) substantially non-identical to said first segment, (ii) substantially identical to a second portion of said specific nucleic acid sequence, (iii) capable of binding to a complementary sequence of said second segment and (iv) capable of providing for subsequent binding of a first segment of a subsequent first primer to said first portion of said specific nucleic acid sequence under isostatic or limited cycling conditions, such that a second primer extension is produced and displaces said first primer extension; and

a second initial primer or a nucleic acid construct complementary to said first primer extension, said second initial primer or nucleic acid construct comprising a segment characterized by capable of template-dependent extension under isostatic or limited cycling conditions; and substrates, buffer and a template-dependent polymerizing enzyme; incubating said specific nucleic acid sequence and said novel primer or nucleic acid construct in the presence of said substrates, buffer and template-dependent polymerizing enzyme under isostatic or limited cycling conditions; thereby non-linearly amplifying said specific nucleic acid sequence.

24. The process of claim 23, wherein at least one member selected from the group consisting of said first segment or said second segment of the first initial primer or nucleic acid construct, said segment of the second initial primer or nucleic acid construct, and said primer extension, or any of the foregoing, comprise at least one modified nucleotide or nucleotide analog.

25. The process of claim 24, wherein the second segment of the first initial primer comprises at least one modified nucleotide or nucleotide analog which increases the thermodynamic stability of said first segment to its complement in said primer extension.

26. The process of claims 24 or 25, wherein said modified nucleotide or nucleotide analog comprises an intercalating agent.

27. The process of claim 23, wherein said first segment of the first initial primer or said segment of the second initial primer, or both, or their primer extension, or

any combination thereof, comprises at least one modified nucleotide or nucleotide analog.

28. The process of claim 27, wherein said modified nucleotide or nucleotide analog decreases the thermodynamic stability of said first segment or said primer extension, or both, to its complement.

29. The process of claim 28, wherein said modified nucleotide or nucleotide analog comprises a negatively charged chemical group.

30. The process of claim 29, wherein said negatively charged chemical group comprises carboxylic acid.

31. The process of claim 23, wherein said first initial primer or nucleic acid construct, or said second initial primer or nucleic acid construct, or both, comprises a nucleic acid selected from the group consisting of a linear nucleic acid, branched nucleic acid, an inverted nucleic acid and a peptide-nucleic acid, or a combination of any of the foregoing.

32. A process for non-linearly amplifying a specific nucleic acid sequence comprising the steps of:

providing:

said specific nucleic acid sequence;

a singular primer or a singular nucleic acid construct capable of non-linear amplification, comprising three segments:

(a) a first segment (i) being substantially complementary to a first portion of said specific nucleic acid sequence and (ii) capable of template-dependent first extension;

(b) a second segment substantially identical to a second portion of said specific nucleic acid sequence; and

(c) a third segment substantially identical to said first segment;

wherein said first primer extension is capable of producing sequences that are capable of hybridizing to said second segment and is capable of self-priming and self-extending to produce a complement to said third segment, and

substrates, buffer and a template-dependent polymerizing enzyme; and

incubating said specific nucleic acid sequence and said primer or nucleic acid construct in the presence of said substrates, buffer and template-dependent polymerizing enzyme; thereby non-linearly amplifying said specific nucleic acid sequence.

33. The process of claim 32, carried out under conditions selected from the group consisting of isostatic conditions, limited cycling conditions and full cycling conditions.

34. The process of claim 32, wherein a member selected from the group consisting of said first segment, said second segment, said third segment, said first primer extension, said second primer extension, or any of the foregoing, comprise at least one modified nucleotide or nucleotide analog.

35. The process of claim 32, wherein said singular primer or nucleic acid construct comprises a nucleic acid selected from the group consisting of a linear nucleic acid, branched nucleic acid, an inverted nucleic acid and a peptide-nucleic acid, or a combination of any of the foregoing.

36. The process of claim 32, wherein a member selected from the group consisting of said first segment, said second segment, said third segment, said first primer extension and said self priming extension, or any combination thereof, comprises at least one modified nucleotide or nucleotide analog.

37. The process of claim 32, wherein said first primer extension is carried out under conditions selected from the group consisting of limited substrate conditions, limited extension duration, or both.

38. The process of any of claims 1, 12, 23 or 32, wherein said specific nucleic acid sequence is in single-stranded or double-stranded form.

39. The process of any of claims 1, 12, 23 or 32, wherein said specific nucleic acid sequence is found or contained in a fragment.

40. The process of claim 39, wherein said fragment is produced by a means selected from the group consisting of physical means, chemical means, physico-chemical means and enzymatic means, or combinations thereof.

41. The process of claim 40, wherein said physical means are selected from the group consisting of sonication and heat, or both.

42. The process of claim 40, wherein said chemical means comprise acid treatment.

43. The process of claim 40, wherein said enzymatic means is carried out by or with nucleases and restriction enzymes.

44. The process of claim 43, wherein said nucleases comprise endonucleases.

45. A post-termination labeling process for nucleic acid sequencing comprising the steps of:

producing, in the presence of untagged or unlabeled substrates, untagged or unlabeled primer, polymerizing enzyme, buffer and an appropriate untagged or unlabeled terminator for each nucleotide base, nucleic acid fragments corresponding to said nucleic acid sequence of interest, wherein each of said terminators comprise a chemically reactive group that covalently binds to a tagged molecule under conditions that internal sequences are substantially non-reactive to said tagged molecules and said chemical reactions do not substantially interfere with separation of said fragments in a medium or matrix;

separating the fragments produced in a medium or matrix; and

detecting said separated fragments by detecting said tagged molecule in said medium or matrix.

46. The process of claim 45, wherein said producing step the chemically reactive groups of said terminators are protected prior to enzymatic incorporation into the fragment produced and deprotected prior to covalently binding any tagged molecule.

47. The process of claim 45, wherein said producing step said chemically reactive group comprises a nitrogen, a sulfur or an oxygen atom.

48. The process of claim 45, wherein said chemically reactive groups on said terminators are different.

49. The process of claim 45, wherein said chemically reactive groups on said terminators are the same.

50. The process of claim 45, wherein said producing step said tagged molecule is the same for each terminator.

51. The process of claim 45, wherein said producing step said tagged molecule is different for each terminator.

52. The process of claim 45, wherein said tagged molecule is selected from the group consisting of fluorescent dyes, chemiluminescent dyes, infra-red dyes, chemiluminescent entities and electrochemiluminescent entities, or combinations thereof.

53. The process of claim 45, wherein said separating step is carried out electrophoretically.

54. The process of claim 45, wherein said separating step the medium or matrix comprises a gel.

55. The process of claim 45, wherein said gel comprises polyacrylamide gel.

56. The process of claim 45, wherein said separating step is carried out by capillary gel electrophoresis.

57. The process of claim 45, wherein said detecting step is carried out by a means selected from photometric measurement, spectrophotometric measurement, colorimetric measurement, fluorometric measurement, delayed fluorescent measurement and chemiluminescent measurement, or combinations thereof.

58. A process for producing nucleic acid sequences that have decreased thermodynamic stability to complementary sequences, said process comprising the step of incorporating into the nucleic acid sequences produced at least one modified nucleotide or nucleotide analog having a negatively charged chemical moiety.

59. A single-stranded or double-stranded nucleic acid polymer selected from the group consisting of a linear nucleic acid, branched nucleic acid, an inverted nucleic acid and a peptide-nucleic acid, or a combination of any of the foregoing, wherein said nucleic acid polymer comprises at least one purine or pyrimidine base comprising one negatively charged chemical moiety in one or both strands.

ADD 7 ADD 7 ***
B2